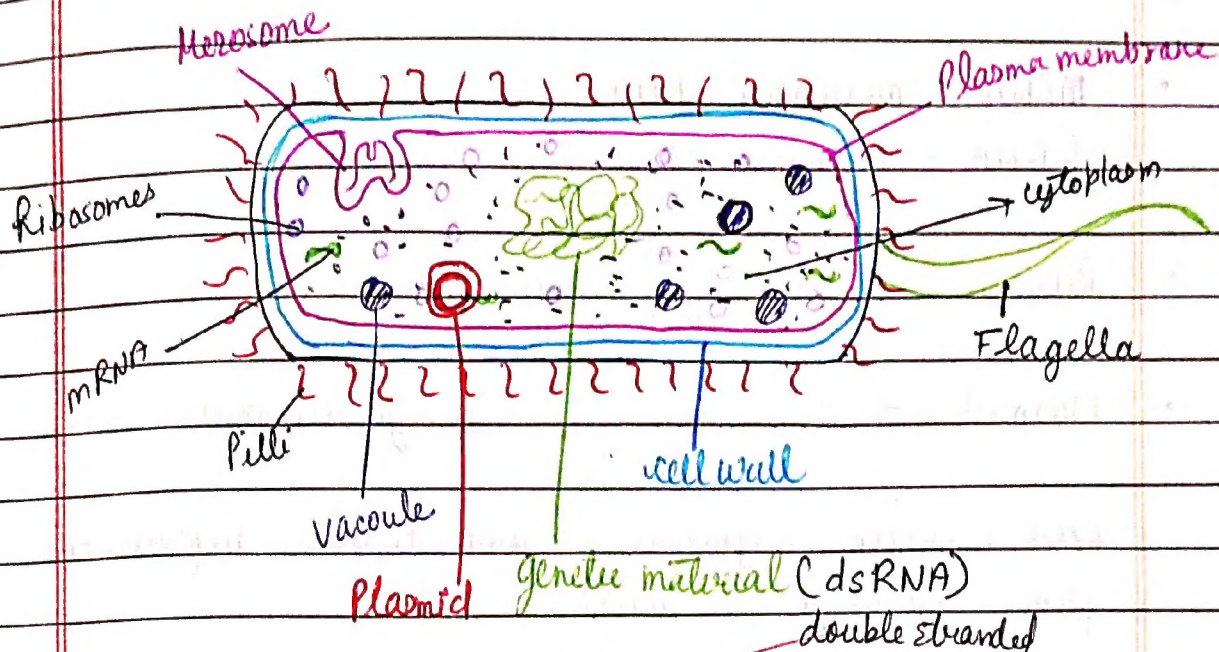


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Structure of Bacterial cell.



- Plasma membrane is semi permeable.
Transport of waste material
- Infolding mesosome
bacterial respiration is takes place by mesosome.
- Flagella - locomotion movement of bacterial cycle.
- Pilli
Thin short hair like Acute ssDNA.
help in attachment to any structure

2/4/22

- Cytoplasm
dense material and provide rigidity to bacterial cell
- Genetic material (dsDNA)
In center
- mRNA - messenger RNA.
tRNA - Transfer RNA.
- Ribosomes - site of protein synthesis
- Plasmid - extra chromosomal genetic material
used genetic engineering and shares information
from bacteria to bacteria.

MICROBIOLOGY

study of microorganism under the microscope

History of Microbiology -

Father of modern Microbiology - Louis Pasteur

Fermentation

sterilisation

Pasturisation

~~Robert Koch~~ - Germ theory of disease.


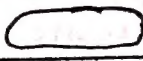


Antonie Van Leeuwenhoek - Compound microscope

Joseph Lister - Antiseptic surgery.

Edward Jenner - vaccine for pox virus.

classification of Microorganism -

On the basis of shape and size.

- | | | | |
|----|----------------|---------------------|--|
| 1 | cocci | eg. streptococcus |  spherical |
| 2 | Bacilli | eg. salmonella |  Rod |
| 3 | Comma (Vibrio) | eg. vibrio cholerae |  comma shaped |
| 4. | Spirilla | eg. spirillum minus |  spiro |

⇒ On the basis of nutrition

Autotrophic bacteria - Bacteria that prepare food by its own.

Heterotrophic bacteria - Bacteria that depends upon other.

1. Autotrophic bacteria

a) Phototropic Bacteria - use sunlight for preparing food.

e.g. - Cyanobacteria, Green bacteria, purple bacteria

b) Chemotropic bacteria - Bacteria use chemical energy for prepare food.

• chemotrops (Inorganic compounds use)

eg. Sulphur bacteria

Nitrifying bacteria

(Nitrosomonas)

i) Chemorganotrops - Bacteria use organic compound

e.g. Purple non-sulphur bacteria.

2. Heterotrophic Bacteria -

↓
Saprophytic
Bacteria

(grow and
dead and decaying
organic matter)
Fungus
Bacillus cereus

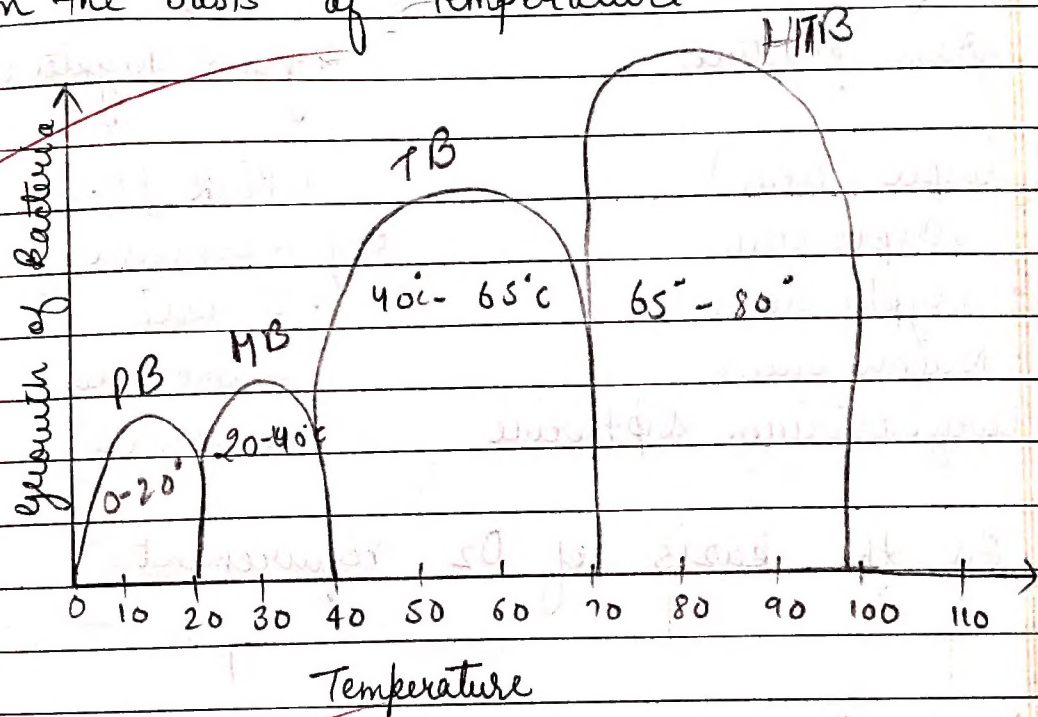
↓
Symbiotic
Bacteria

(do nitrogen
fixation,
mutual association
with plants)
Eg. Rhizobium.

↓
Parasitic
bacteria

(disease causing
bacteria)
Eg. Salmonella.

⇒ On the basis of Temperature -



0-20 - Psychrophilic bacteria

20-40 - Mesophilic bacteria

45-60/65 - Thermophilic bacteria

65/70 - 80/90 - Hyperthermophilic bacteria

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⇒ On the basis of pH (0-14)

Acidic

0 - 7 0.65 - Acidophilic bacteria eg. Lactobacillus

7-neutral 6.5 - 7.5 - Neutrophilic eg. E-coli

7-14 8 - 14 - Basophilic / Basilophilic bacteria eg. Vibrio cholerae
Basic.

⇒ On the basis of staining

↓
gram positive

(Purple / Blue)

eg. Streptococcus

• Staphylococcus

• Neumococcus

(Rod) Corynebacterium diphtheriae

↓
gram negative

(Pink / Red)

e.g. Neisseria (cocci)

• E-coli

• Salmonella

• (Neisser's)

⇒ On the basis of O₂ requirement

Aerobic bacteria

Requires oxygen

Two types -

Obligate aerobes - eg. Vibrio cholerae

which grow only in presence of O₂

Microaerophilic bacteria - are those

grow best in the presence of low

O₂ tension eg. Helicobacter

Anaerobic bacteria

grow in absence of O₂

Facultative anaerobes - O₂ presence


absence) Microaerotolerant

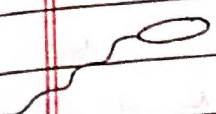
bacteria (If we give


them 5-10% O₂ they

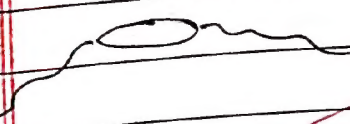
can also grow).

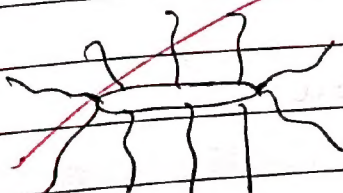
4. On the Basis of flagella

a)  - Atrichous - no flagella eg. Bacillus

b)  - Monotrichous - eg. Cholera vibrios

c)  - Lophotrichous eg. Pseudomonas.

d)  - Amphitrichous eg. spirillum

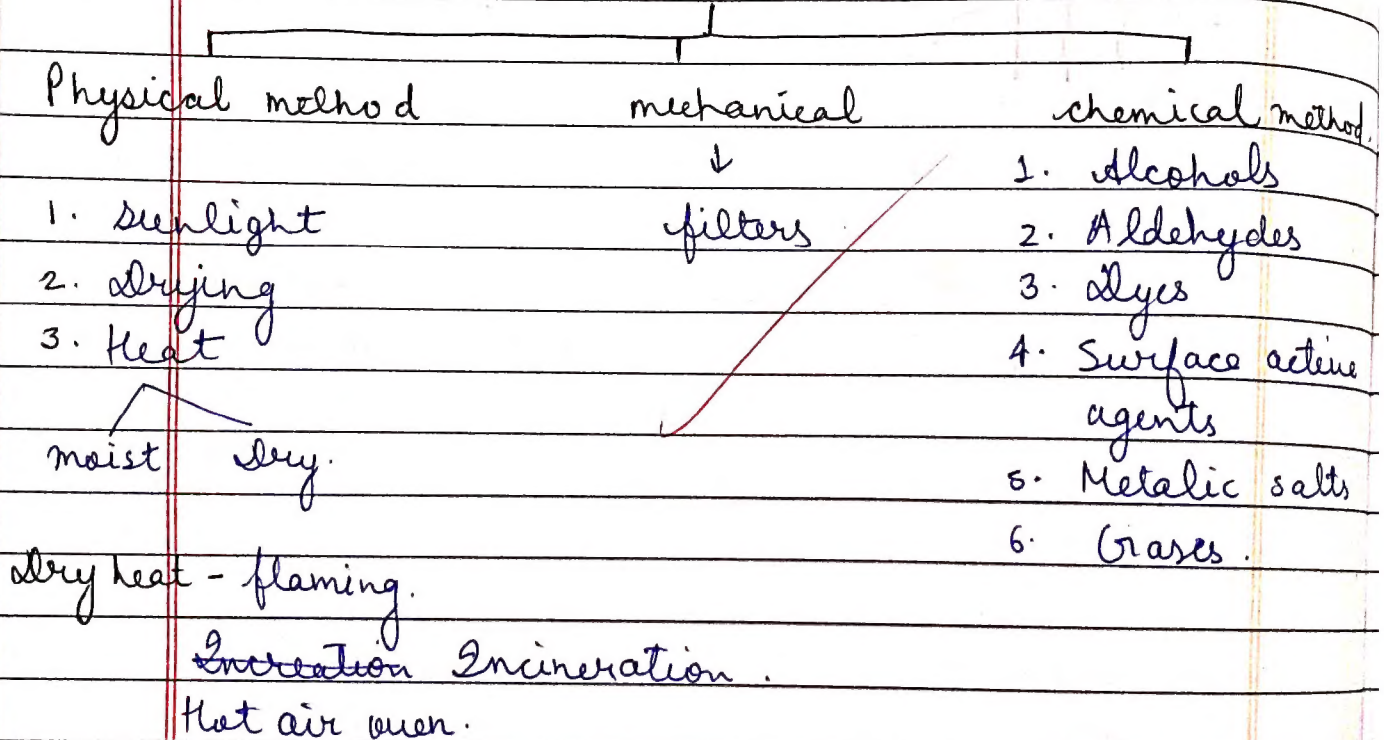
e)  - Peritrichous eg. E-coli, Salmonella

STERILIZATION

The term sterilization is defined as the process by means of which an article surface medium is made free from all living microorganisms including spores.

sterilisation is the complete inactivation of all forms of microbial life.

Sterilizing agent
Physical, chemical.

Sterilisation Method.

Moist heat - 1) Pasteurization

- 2) Boiling
- 3) Autoclave

chemical method and their agents.

1) Alcohols - Ethyl alcohol

2) Aldehydes - Formaldehydes

3) Dyes - Acridine dyes, Aniline dyes.

4) Surface active agents - Soap, detergents

5) Metallic salts - Mercuric chloride.

6) Gases - Ethylene oxide, formaldehyde.

Mechanical method



Filters

membrane filter

chamberland filter.

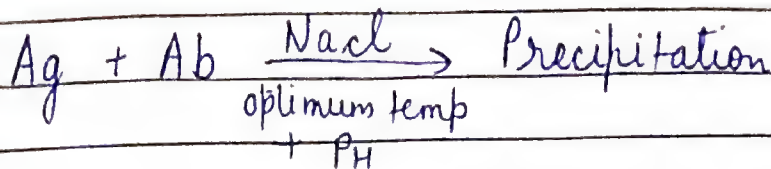
Disinfection

The term ~~disinfection~~ is defined as the process of ~~destruction~~ of vegetative forms of pathogenic organisms which are capable of producing infection but not necessary.

Disinfection : are usually reserved for inanimate objects.

Antiseptics
Antiseptics are the chemical substance can be safe chemical disinfectant to skin/mucous membrane and are used to prevent infection by inhibiting the growth of bacteria.

Chapter - 13

Antigen - Antibody Reactions
Serological ReactionsPrecipitation Reaction

When a soluble antigen combines with its antibody in the presence of electrolytes (NaCl) at a suitable temperature and pH, the antigen-antibody complex forms an insoluble precipitate.

Ag - Ab Reaction's / Serological Reactions -

Serological is the study of serum it is the science that studies antigen antibody reactions of the body using a serum specimen.

Many diagnostic laboratory test are based on antigen antibody reactions. These test to detect antibodies against the infecting microorganism provide a useful means of indirect diagnosis.

These test are of special value for those organisms which cannot be isolated and cultured in the laboratory.

Serological reactions are simple rapid and specific

The test results are reliable and reproducible.

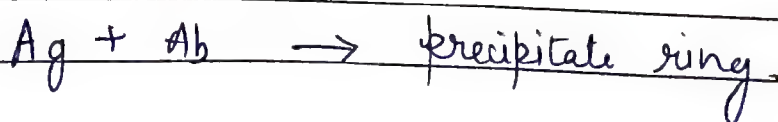
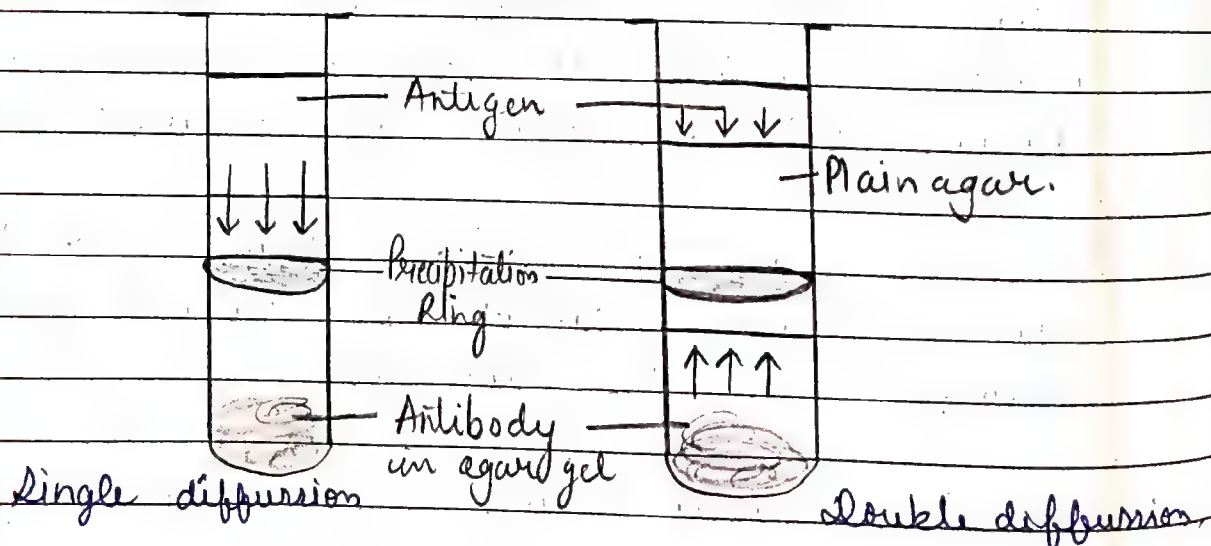
Types of Serological Reactions -

- a. **Precipitation Reaction** - When antigen and antibody reaction with each other in the presence of NaCl and optimum temp & pH it results into precipitation reaction.

b. Types of slide precipitation Reaction -

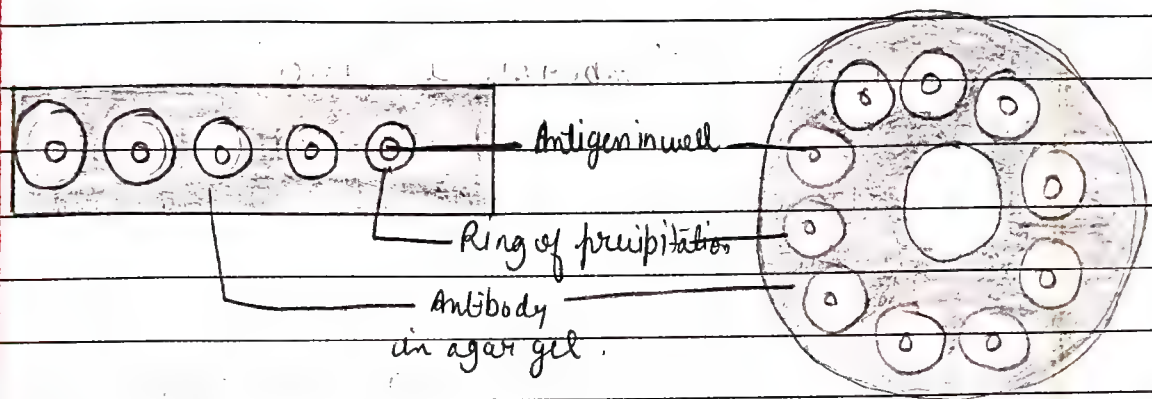
- i) **Test Tube precipitation Reaction** - The antibody is incorporated in gel, above which is placed in a column of plain agar. The antigen is layered on top of this. [Double Diffusion]

Ag & Ab move towards each other through intervening column of plain agar and form a band of precipitate at point where they meet at optimum proportion.



b) Single diffusion - The ab is incorporated into agar gel in a test tube and the antigen solution is layered over it. The antigen diffuses downward through the agar gel forming a line of precipitation.

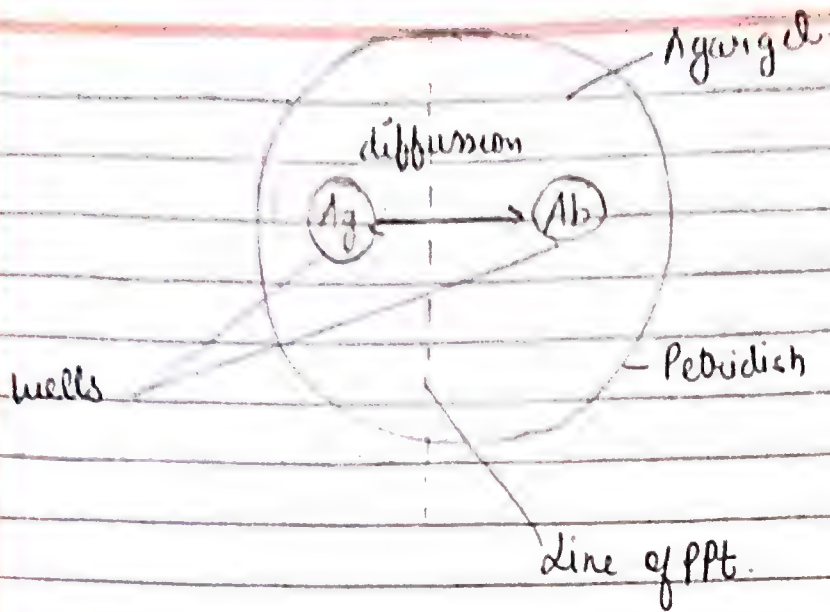
Immunodiffusion Reaction - Antiserum is incorporated in agar gel poured on a flat surface like slide. The antigen is added to the wells on the surface of the gel. It diffuses radially from the well and forms ring-shaped bands of precipitation, concentrically around the well.



b) Double diffusion in 2-D -

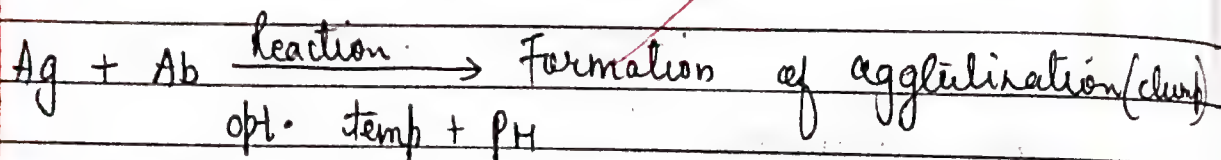
Agar gel is poured on a slide and wells are cut using a template. The antiserum is placed in the central well, & different antigens in the surrounding wells.

If two adjacent antigens are identical, the lines of precipitate formed by them will fuse and if they are ~~identical~~ non-identical the LOP will cross each other and indicated by spur formation.



2) AGGLOUTINATION REACTION:

When a particulate antigen mixed with the its antibody in the presence of electrolytes at a suitable temperature and pH, the particles are clumped or agglutinated. This reaction is known as agglutination reaction.



eg- Blood grouping slide test.

Agglutination occurs only when antigen and ~~ab~~ antibody are mixed ~~with each other~~ at optimal proportions.

NEUTRALISATION REACTION.

When an antitoxin combines with the a toxin the biological effects of the toxin are neutralised rendering it harmless to animals since toxin is an antigen in solution. It is also precipitated.

Toxin, Anti-Toxin Neutralization can be measured in vivo (in) and in vitro (in).

Virus neutralisation Test - Neutralising coat viruses once coated the viruses cannot penetrate cells in the body. Test serum is mixed with a suspension of infectious virus particles of the same type as that of the individual is suspected to have been infected.

The virus suspensions are then inoculated into amputated at or tissue culture that supports the growth of virus.

1. Neutralisation test in IV - (In vitro) [Schick Test]
Agar gel in the precipitation.
It is employed to detect the production of toxins by Corynebacterium diphtheriae.

2. Anti-Streptolysin O (ASO) test - It demonstrates that the antitoxin present in serum of patient infected with streptococcus contains Anti-streptolysin O, that neutralises the streptolysin O.

immunosorbent - an absorbing material specific for one of the components of the reaction (Ag-Ab) for ~~diagnosis~~ ^{cellulose / agarose, cellophane / polystyrene}. (Date: / / 201)

ELISA - Enzyme Linked Immunosorbent Assay
Based on Ag-Ab reaction.

Principle - It is plate based immuno assay. It works on the principle of antigen-antibody binding. It is based on enzymatic colour reaction. It detects and identifies substances such as peptides, proteins, hormones and antibodies.

Sample for ELISA -

1. Serum
2. CSF
3. Sputum
4. Urine
5. Stool

Types of ELISA

1. Direct ELISA
2. Indirect ELISA
3. Sandwich ELISA

1. ~~Direct~~ Direct ELISA - (~~Direct~~ direct ELISA).

Conjug
is was
out as
not free

direct ELISA

Microtiter well coated with Antigen



And serum (contain primary antibody that is enzyme linked)



wash - To remove unbound antibodies



Add substrate (chromogen) for the enzyme

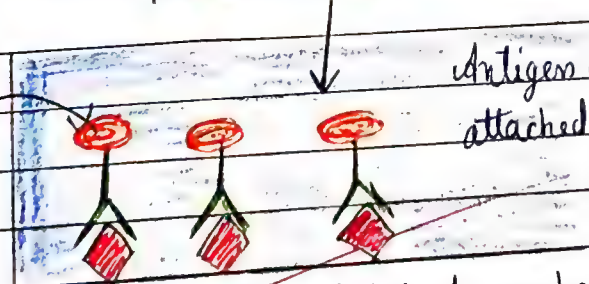


Enzyme cleaves substrate to produce color

Virus sample on the surface.

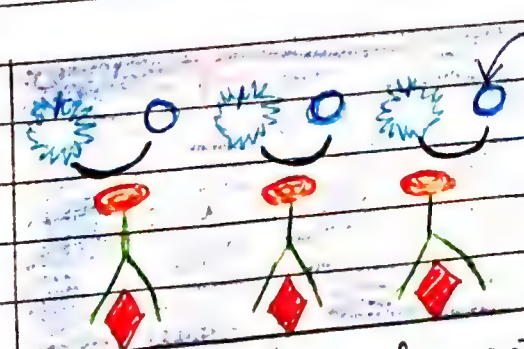


Conjugate is washed out as ag is not free to bind the conjugate



Antigen with enzyme conjugate attached to viral antigen

Substrate and enzyme interaction create color change for detection.



substrate

Enzyme is not there to act on substrate

No colour (negative)

If it is +ve

Produces phosphatase as

Indirect ELISA

Microtiter well coated with Antigen



Add serum (contains primary Ab)



Wash - To remove unbound antibodies



Add secondary Ab (Enzyme linked)



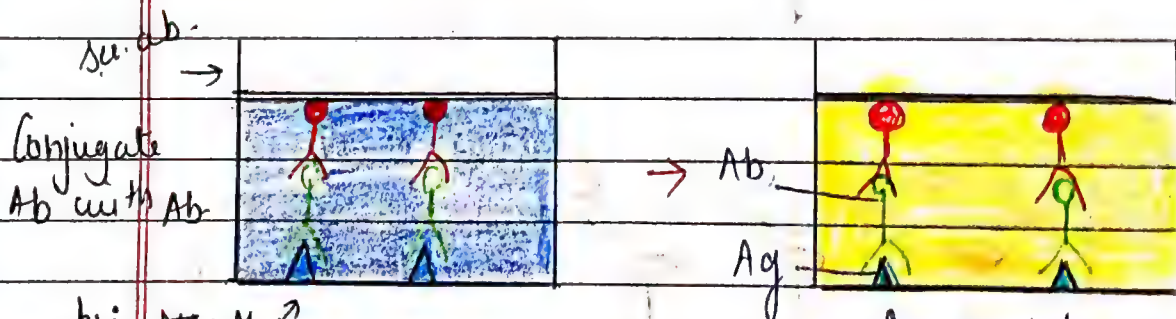
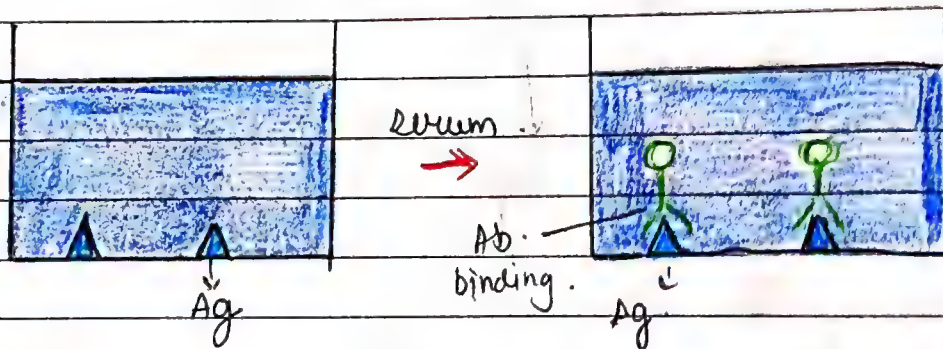
Wash - To remove unbound secondary Ab



Add substrate (chromogen) for the enzyme.



Enzyme cleaves substrate to produce color.



pri Ab →

colour (positive) test changed

Conjugate Ab → A
→

Sandwich ELISA

Microtiter well coated with capture Antibody



Add serum (contains Ag to be detected)



Wash to remove unbound antibodies



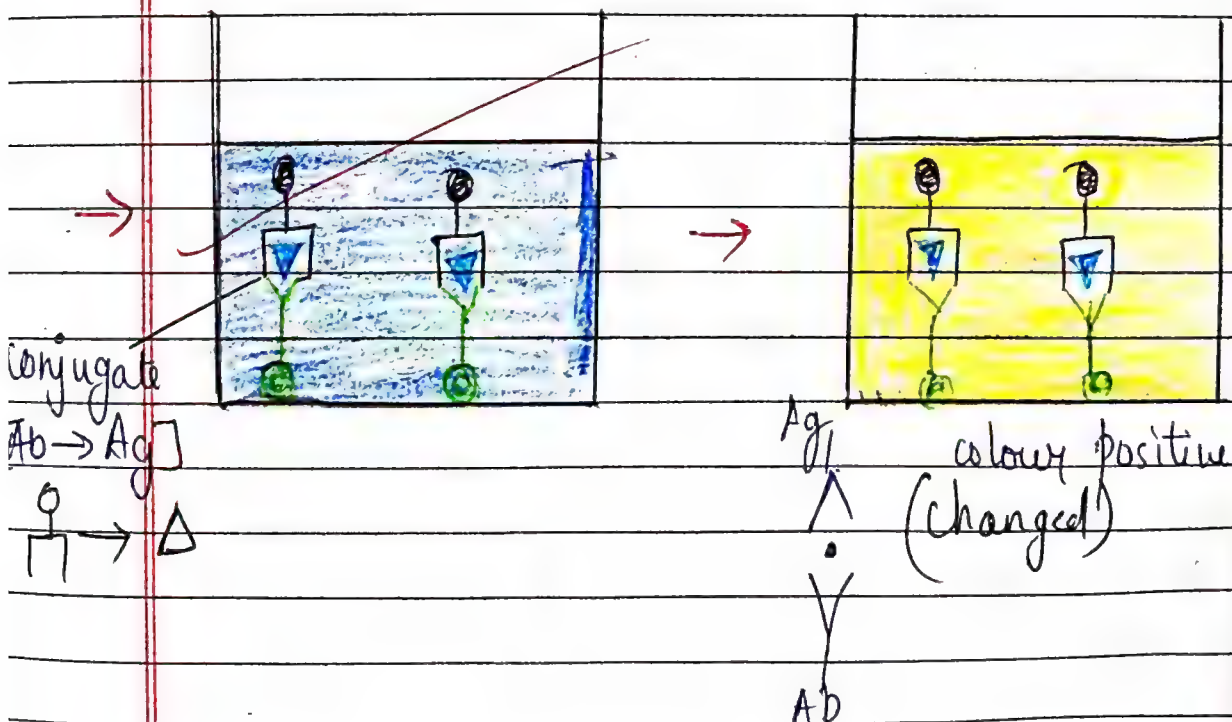
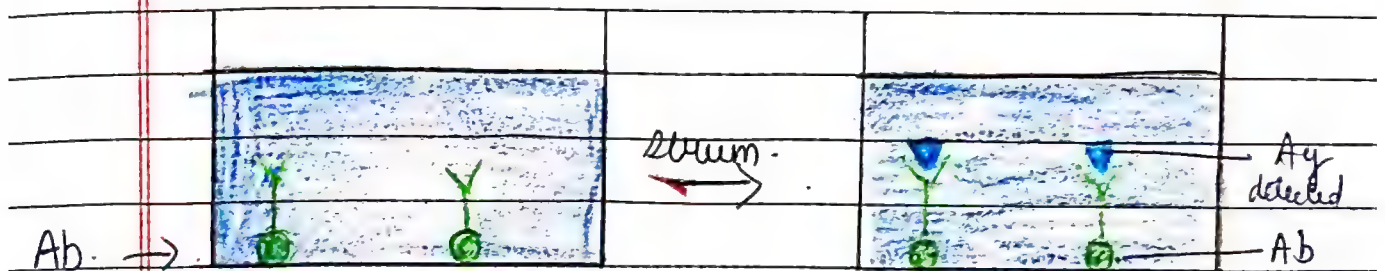
Add enzyme linked antibody



Add substrate (chromogen) for the enzyme



Enzyme cleaves substrate to produce color



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STAPHYLOCOCCUS

It is a gram positive cocci occurring in groups like cluster, because there is a cell division takes place in all planes. It is a hemolytic cocci.

It is a commonest positive organism of pyogenic lesions in men.

They are catalase positive aerobes or facultative anaerobes.

Grow in presence of 7.5% - 10% NaCl

These are spherical, unid, gram positive cocci. Their size approximately measure 1 μ m in diameter.

Source of infection -

Human patient and carrier due to are to commonest source of infection in hospital more than 50% of the hospital staff is carriers.

Staphylococcus food poisoning mostly occurs due to the contamination of food from food handlers.

Pathogenesis -

1. S. aureus - The main pathogen causing pyogenic causes in men.

2. *S. epidermidis* - It may lead to septicemia and subacute endocarditis.
It may produce minor lesion like stich, abscess.
3. *S. Saprophyticus* - mainly seen in genito urinary mucus membrane and skin
It act as opportunistic pathogen

Cultural characteristics -

S. aureus - grows easily and optimum easily temperature of 37°C and pH of 7.4 in the presence of O_2 .

In nutrient agar media on 24h incubation produces golden yellow circular convex smooth 2-4 mm shiny and opaque colonies.

In Macconkey media - colonies are small and pink.

Lab diagnosis -

Sample - blood.

Gram staining - appear blue / purple cocci in cluster.

Culture -

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STREPTOCOCCUS / STREPTOCOCCI

Streptococci are gram positive arranged in chains or pairs.

Characteristics -

1. These are gram positive.
2. Non-sporing
3. Non-mobile
4. Capsulated (lead to more pathogenic)
5. Aerobic & normally facultative anaerobic (C can be grow both in absence / presence of oxygen)
6. The individual cocci are spherical or cuboid approximately measure $0.5 - 1 \mu m$ in diameter and characteristically arranged in chains varying in length.

Source of Infection -

Human patient & carriers are the commonest source of infection.

Streptococcus infection are less frequent in children less than 3 years old but more frequent in school age children.

Nasal carriers are the most dangerous source of the organism.

Pathogenesis -

Acute infection of respiratory tract -

- i) Streptococcal sore throat - This is the most common disease, may be localised as acute tonsillitis or may involve the pharynx more diffusely.
- ii) skin infection - Empetigo & pyoderma manifest as localised infections with induration tenderness & pus point.
- iii) Acute Tonsillitis
- iv) Sepsis
Seplicemia.

Abscess in internal organ.

Bacteremia - infection in blood.

B. Toxin mediated infection

i) Scarlet fever

ii) Toxic shock syndrome.

C. Non Suppurative infectious sequelae.

• Rheumatic fever

Hypersensitivity reactions.

Biochemical Reactions -

Most sugar like glucose, lactose, sucrose, maltose, manitol are fermented & production of acid takes place but no gas.

They are catalase negative.

Streptococcal Diseases.

Group A

Lesions

Streptococcal Pyogenes

Suppurative Infections

- a) Respiratory Tract
- b) Skin and soft tissue manipulation

Sore throat, tonsillitis, impetigo, pyoderma, erysipelas, infection of wound, burns & chronic skin infections like eczema, psoriasis.

- c) Central infections
- d) Deep infections

Puerperal sepsis, Bone & joint infections, lymphadenitis, septicaemia, acute endocarditis.

Non-Suppurative Lesions.

- a) Heart
- b) Kidney

Rheumatic Fever, Acute post streptococcal glomerulonephritis, Endocarditis, urinary tract infection, Endocarditis.

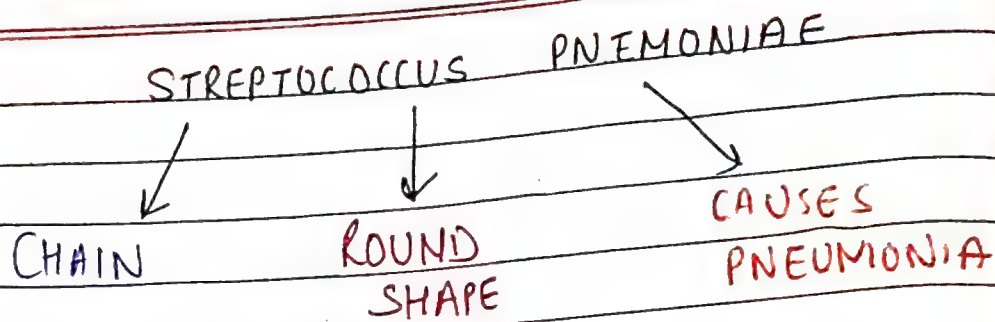
Streptococcal faecalis
Streptococcal faecium
Streptococci viridans

Group B streptococci

Neonatal infections, C. pneumonia, meningitis, respiratory disease.

Medically relevant streptococci -

| species | Host | Disease. |
|---|------------------|---|
| S. Pyogenes Scarlet fever. Erysipelas | Human | Pharyngitis cellulitis erysipelas. |
| S. agalactiae | Human cattle | Neonatal meningitis & sepsis. |
| S. dysgalactiae | Human animals | endocarditis. bacteremia. pneumonia, meningitis. respiratory work infections. |
| S. gallolyticus | human animals | biliary or urinary tract infections. endocarditis. |
| S. anginosus | human animals | subcutaneous / organ abscesses meningitis. respiratory infections. |
| S. sanguinis | human | endocarditis, dental caries. |
| S. suis | pig | meningitis. |
| S. mitis | human | endocarditis. |
| S. mutans | human | dental caries. |



— Round Bacteria

grow in chains

Most common cause of — community acquired pneumonia

Pneumonia symptoms.

→ Fever

→ Labored breathing

→ Cough

→ Fever

→ chills

→ loss of appetite

→ Nausea & vomiting especially in small children

→ ~~shape~~ rise in temp

→ chest ~~in~~ pain when coughing

→ Severe weakness.

→ Bluish skin

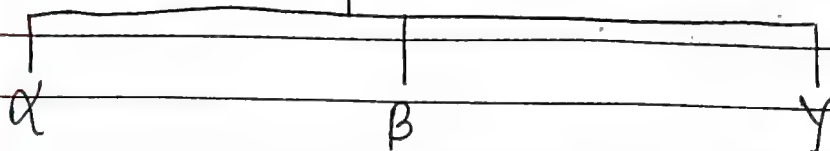
→ Body aches.

→ Meningitis associated with pneumonia

STREPTOCOCCUS



gram positive cocci in chains, catalase negative



Partial hemolysis

Complete hemolysis

No hemolysis

optochin

sensitivity

complete hemolysis

complete hemolysis

grow 6.5% NaCl

No

Yes

viridans
Strep
(unencapsulated)

S. pneumoniae
(encapsulated)

No

Yes

S. bovis

Enterococci

S. faecium

S. faecalis

No

Yes

group B

group A Strep

S. agalactiae

S. pyogenes

exially

coughing

with

Laboratory Diagnosis.

Sample - 1) **Blood sample.**

2) **Sputum.**

3) **urine.**

4) **CSF.**

steps -

1. **direct microscopic examination** - of bacterial smear. - after smearing the sample is forwarded by gram staining and as a result blue colour coccus appears in chain like structure.

2. **culture - Nutrient agar media.**

after 24 hours incubation the colonies are large (2-4 mm diameter), round smooth shiny and often pigmented. staphylococcal pigment is believed to be a carotenoid and its derivative located in cell membrane.

Most strain form the golden yellow pigment or orange yellow pigment colonies.

2. **Blood Agar Media -**

colonies are almost similar to those on nutrient agar. They produce beta haemolysis which is best seen with rabbit or sheep blood.

3) **MacConkey's Agar** - colonies are very small and pink due to lactose fermentation.

4 ~~Bio~~ Biochemical Reactions / Test

They ferment sugar and produce acid but no gas.

They are catalyse positive
Reduce nitrates to nitrites ($\text{NO}_3 \rightarrow \text{NO}_2$)

Prevention for infection.

?

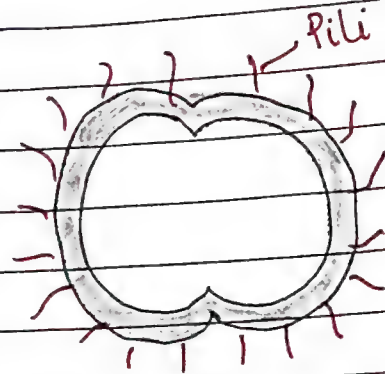
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(Date / 201)

Neisseria (gram -ve cocci)

Neisseria
Meningitis

Neisseria
gonorrhoea
(gonorrhoea)



Neisseria Meningitis -

These are gram negative diplococci commonly colonize the nasopharynx of healthy people. It was first isolated by Weichselbaum in 1887 from spinal fluid of a case of meningitis. It causes acute purulent meningitis & also called as cerebrospinal meningitis or cerebral fever.

characteristics -

These are gram negative oval cocci. $0.6 - 0.8 \mu\text{m}$ occur typically in pairs with adjacent sides flat or concave bean shaped and the axes of the pair parallel and not in line as in the pneumococcus.

These are non-motile and capsulated.
Source of infection -

- Human patients are the commonest carriers.
Nasal carriers are the most dangerous source of the mechanism.

Pathogenesis -

Meningococemia presents with acute fever, chill, cutaneous rash, Hemorrhages often associated with metastatic involvement of joint, ears, lungs & myocardium.

Diagnosis -

a) Specimen and sample

→ CSF or Blood.

1st process detection of organism by smear examination and immunological examination.

2nd process

b) Biochemical Test - Catalase and oxidase positive ferment glucose and maltose producing acid but no gas.

c) Culture - Grows well media enriched with blood serum or specially in presence of 10% CO₂.
colonies in solid media are moist, elevated, round, convex and translucent.

colonised in solid media are moist, smooth, elevated, brown, convex and translucent.

Examination - Direct

- 1 gram staining
- 2 growth in chocolate agar medium and higher Martin selective media.
- 3 observing fermentation of glucose
- 4 oxidative test positive

Indirect examination

- 1 Complement fixation Test
- 2 Fluorescent Ab. Technique.
- 3 ELISA
- 4 Mucocytosis.

27/05/22

Causes - 'Wahaguru ji'
Tb Causes - Mycobacterium tuberculosis
Known as - Acid Fast bacteria

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GRAM POSITIVE BACILLI

(appears red after staining)

Diag - Acid fast staining and Ziehl Neelsen staining.

TUBERCULE BACILLI

Mycobacterium tuberculosis is also known as Tubercule Bacilli. It is a pathogenic bacterial species in the genus mycobacterium and causative agent of Tuberculosis.

First discovered on 24th March 1882 by Robert Koch who received the noble prize in medicine for this discovery in 1905.

The bacterium is also known as 'Koch Bacillus'.

characteristics -

1. It is a non-motile, non-sporing, non-capsulated bacillus measures $2-3 \times 0.2 - 0.4 \mu m$. Arranged singly or in groups.
2. This is an obligate aerobe (only grow in the presence of oxygen) hence acid fast staining is used for their staining.

Source of infection -

Mycobacterium tuberculosis cause human lesion like pulmonary Tuberculosis.

Infection in human is transmitted mainly by inhalation of bacilli in moist droplets of respiratory secretions from the open pulmonary tuberculosis.

occasionally infection occurs by ingestion of infected milk (cow) Mycobacterium - Bovis found in milk of cow.

The bacilli are more resistant to drying and chemical disinfectants. Moist heat at 100°C kills them readily. Its unusual cell wall rich in lipids (**Mycolic acids**) is likely responsible for this resistant

cultural characteristics - These are aerobes, grow slowly colonies usually appear in 2-3 weeks and may some times require 2 weeks incubation with optimum temperature 37°C at pH 6.4-7.0. They grow only in special enriched media (**additional nutrient added**) containing eggs, potatoes, serum and meat extract. **Gordonstein Jensen (L.J Medium)** is used for their cultivation.

Pathogenicity -

Primary infection - Primary infection may occur in any organ like lungs, tonsils, skin or intestine. In children the usual site is in the lungs. In most cases the primary infection is asymptomatic & undergoes spontaneous clearing by resolution.

Distinguishing b/w M. Tuberculosis & M. Bovis

| Test | M. Tuberculosis |
|----------------------------------|---|
| 1. Morphology | Slender, long and usually curved |
| 2. Staining | Barred or beaded |
| 3. Growth | Eugonic |
| 4. Action of glycerol in media | Growth enhanced. |
| 5. Colony | Dry rough raised, wrinkled Colour - creamy white / buff colour |
| 6. Biochemical Reaction | |
| i) Nicotin production | positive |
| ii) Nitrate reduction | positive |
| iii) TCH selective action | negative. |
| 7. Animal Pathogenicity | |
| i) Progressive disease in Rabbit | negative or mild lesion |
| ii) In guinea pig. | positive progressive & ultimately fatal disease |

Drug concentrations in antimicrobial susceptibility testing for mycobacteria.

| Drug | Lowenstein-Jensen | Middlebrook 7H10 |
|--------------|-------------------|------------------|
| Isoniazid | 0.2, 1 | 0.2, 1 |
| Streptomycin | 2 | 4 |
| Rifampin | 1 | 40 |
| Ethambutol | 2 | 2 |
| Ethionamide | 5 | 20 |
| Kanamycin | 5 | 20 |
| Capreomycin | 10 | 20 |
| Cycloserine | 20 | 30 |
| Pyrazinamide | 50 | |

Solid M

Egg

1) Low

2) WJ

3) Wa

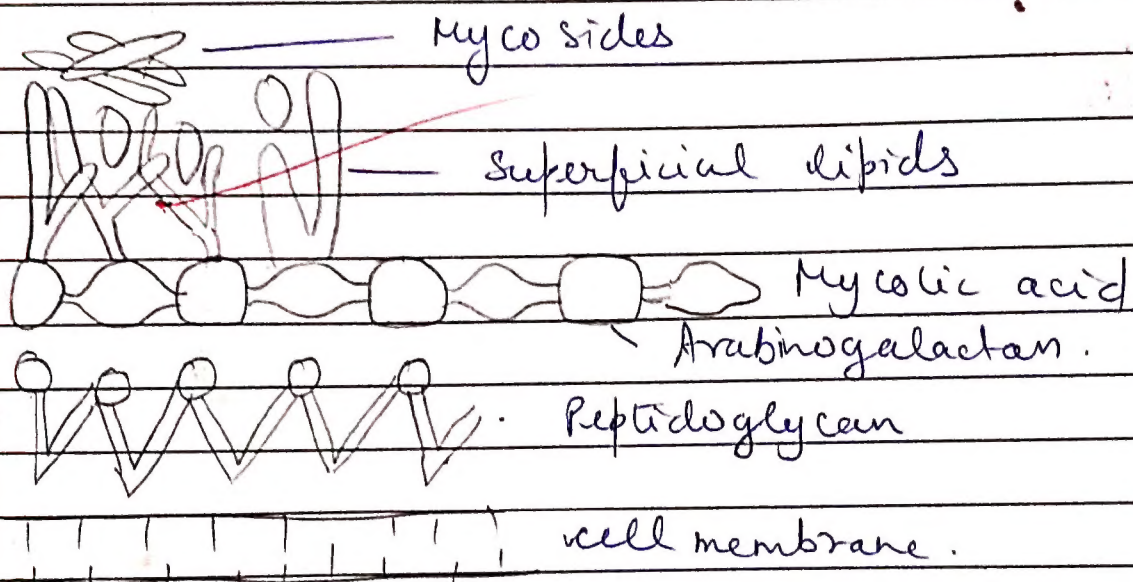
4) Fibr

Identification

staining - Mycobacteria are acid fast (means they stain red/pink) because of the presence of N-glycol-muramic (mycolic) acid in their cell walls.

Ziehl-Neelsen stain is most commonly employed acid fast technique

cell wall of myobacterial cell.



Media for Mycobacterial culture specimen.

Solid Medium

Components

Egg based

- 1) Lowenstein Jensen (LJ) - eggs, defined salts, glycerol
- 2) LJ with pyruvic acid - LJ + pyruvic acid.
- 3) Wallerstein - Egg yolk, 2.5% glycerine.
- 4) Tuberculari - Coagulated eggs, egg yolk whole milk.

Agar-based -

- 1) Middlebrook 7H10 - Salts, oleic acid, albumin, catalase, glycerol
- 2) Middlebrook 7H11 - MB 7H10 + Coarbin

Liquid -

1. Dubo's medium - Boune serum albumin, asparagine, casein, hydrolyzed casein salts with Tween.
2. Middlebrook 7H9 -

20/05/22